Methods: nt-mESCs were cultured using a bioreactor system to develop embryoid bodies, which were induced with 1% ascorbic acid to differentiate into cardiomyocytes. nt-mESC-derived cardiomyocytes (nt-mESCs-CMs) were enriched by Percoll density gradient separation to generate nt-mESCs-PE-CMs. Ischemia was induced by ligating the left anterior descending coronary artery in female Sprague-Dawley rats. Immunosuppressed rats were randomly assigned to received an injection containing 5×10^6 cells of mESCs, nt-mESCs, nt-mESC-CMs, or nt-mESC-PE-CMs.
Results: Analysis performed 8 weeks after transplantation revealed teratoma formation in 80%, 87%, and 33%* of rats administered mESCs, nt-mESCs, and nt-mESC-CMs, respectively, (*P< 0.05 nt-mESC-CMs vs. mESCs). Mean tumor volumes were 82.72±6.52, 83.17±3.58, and 50.50±5.98* mm^3, respectively, (*P< 0.05 nt-mESC-CMs vs. mESCs). In contrast, no teratoma was detected in rats that received nt-mESC-PE-CMs. Octamer-binding transcription factor 4, a specific marker of undifferentiated nt-ESCs, was detected by polymerase chain reaction in rats that received nt-mESCs and nt-mESC-CMs, but not in rats that received nt-mESC-PE-CMs.
Conclusions: nt-mESCs have the same pluripotency as mESCs and teratoma formation with nt-mESC transplantation could be induced by cell differentiation and enrichment.

GW25-e2195

Effect of Regulatory Factors of the Myocardial Mitochondrial Biogenesis of Rats after Acute Exhaustive Exercise at Different Time

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Objectives: Peroxisome proliferator-activated receptor γ, coactivator 1 α (PGC-1α), Nuclear respiratory factors NRF-1 and NRF-2 are the key factors of the regulation of mitochondrial biogenesis. Through the establishment of a single bout of exhausted swimming model, our work is to research the protein and gene expression of mitochondrial biogenesis related factors, PGC-1α, NRF-1 and NRF-2 at different time after exhaustive exercise. In order to investigate the effects of exhaustive exercise on mitochondrial biogenesis of rats after acute exhaustive at different time.
Methods: 1 A total of 40 health male Sprague-Dawley rats (average weight (150 ± 20g)) were randomly divided into 5 groups (n=8 in each group), including sedentary control group, exhausted exercise groups (0, 6, 12 and 24 hours after exhausted exercise). Exhaustive exercise model is a single bout of exhausted swimming model, according to Thomas standards. 2 By ELISA kit to detect lactate dehydrogenase (LDH), creatine kinase isoenzyme (CK-MB), troponin (CTN-I) in serum, to verify the extent of myocardial damage by Exhaustive exercise.3 Using fluorescence quantitative polymerase chain reaction (PCR) to detect the gene expression of PGC-1α, NRF1 and NRF2 each group rats myocardial.4 Western blot was used to detect the protein expression of PGC-1α, NRF-1 and NRF-2.
Results: (1) Compared with control group, level of LDH (U/L), CK-MB (ng/ml), CTN-I (pg/ml) in the blood serum of the 0h, 6h, 12h and 24h after exhausted exercise are Significantly higher (P<0.05). And the level of LDH, CTN-I in the blood serum of the 12h after exhausted exercise is the highest, but the level of CK-MB in the blood serum of the 6h after exhausted exercise is the highest. (2) The gene expression of PGC-1α, NRF-1 and NRF-2 of other groups were significantly higher than that of control group (P<0.05), and (P<0.05). And the gene expression of PGC-1α had a decreasing trend with time. The gene expression of NRF-1 and NRF-2 of 6h after exhausted exercise is the highest. (3) Compared with control group, level of the protein expression of PGC-1α in rats myocardial of 0h, 6h, 12h and 24h after exhausted exercise are Significantly lower (P<0.05). And level of the protein expression of NRF-1 in rats myocardial of 0h, 6h and 24h after exhausted exercise are significantly lower (P<0.05). And level of the protein expression of NRF-2 in rats myocardial of 0h, 6h, 24h after exhausted exercise are Significantly lower (P<0.05).
Conclusions: (1) Acute exhaustive exercise cause myocardial damge. (2) Exhaustive exercise can promote mitochondrial biogenesis through stimulating the expression of PGC-1α, NRF-1, NRF-2 mRNA. (3) The expression of PGC-1α, NRF-1, NRF-2 mRNA after exhaustive exercise has a downward trend, indicating that mitochondrial biogenesis is the performance of rats in the stress response stimulation, the cumulative effect of the mRNA expression does not occur with time. (4) Exhaustive exercise may inhibit PGC-1α, NRF-1 and NRF-2's protein expression.

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Effect of Salidroside on the Regulatory Factors of Myocardial Mitochondrial Biogenesis of Rats after Acute Exhaustive Exercise

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Objectives: Our work is to research the protein and gene expression of mitochondrial biogenesis regulatory factors, PGC-1α, NRF-1 and NRF-2, after exhaustive exercise. In order to investigate the effect of Salidroside on the myocardial mitochondrial biogenesis of rats after acute exhaustive.
Methods: (1) A total of 56 Sprague-Dawley rats were randomly divided into 7 groups (n=8 in each group), including sedentary control group, exhausted exercise groups (0 and 24 hours after exhausted exercise), low-dose SAL and exhausted exercise groups (0 and 24 hours after exhausted exercise), high-dose SAL and exhausted exercise groups (0 and 24 hours after exhausted exercise). Sedentary control group and exhausted exercise groups were administered with saline intragastrically for 14 days. Low-dose SAL and exhausted exercise groups and high-dose SAL and exhausted exercise groups were administered with low-dose SAL (100mg/Kg) or high-dose SAL (300mg/Kg) intragastrically for 14 days. Then exhausted exercise model was established. Exhaustive exercise model is a single bout of exhausted swimming model, according to Thomas standards. (2) By ELISA kit to detect lactate dehydrogenase (LDH), creatine kinase isoenzyme (CK-MB), troponin (CTN-I) in serum, to verify the extent of myocardial damage by Exhaustive exercise. (3) Using fluorescence quantitative polymerase chain reaction (PCR) to detect the gene expression of PGC-1α, NRF1 and NRF2 each group rats myocardial. (4) Western blot was used to detect the protein expression of PGC-1α, NRF-1 and NRF-2.
Results: (1) Compared with control group, level of LDH (U/L), CK-MB (ng/ml) and CTN-I (pg/ml) in the blood serum of the 0h and 24h after exhausted exercise are Significantly higher (P<0.05). and high-dose SAL groups was significantly lower than that of exhaustive group (P<0.05). (2) The gene expression of PGC-1α of other groups were significantly higher than that of control group (P<0.05), and medication group was significantly higher than that of exhaustive group (P<0.05). The gene expression of NRF-1 of other groups were significantly higher than that of control group (P<0.05), and high-dose SAL groups was significantly higher than that of exhaustive group (P<0.05). In addition to exhaustive 24h group after exhausted exercise, the gene expression of NRF-2 of other groups were significantly higher than that of control group (P<0.05), and medication group was significantly higher than that of exhaustive group (P<0.05). (3) Compared with control group, level of the protein expression of PGC-1α and NRF-2 in rats myocardial of other groups are Significantly higher (P<0.05). and high-dose SAL groups was significantly higher than that of exhaustive group (P<0.05). Compared with control group, level of the protein expression of NRF-1 in rats myocardial of the low-dose SAL 24h after exhausted exercise are Significantly higher (P<0.05). 0h and 24h after exhausted exercise, low-dose SAL and 0h after exhausted exercise, high-dose SAL and 0h and 24h after exhausted exercise are Significantly lower (P<0.05).
Conclusions: (1) Acute exhaustive exercise cause myocardial damge. High doses of Salidroside can prevent myocardial damge. (2) High doses Salidroside can promote the protein and gene expression of mitochondrial biogenesis regulatory factors, PGC-1α, NRF-1 and NRF-2 of rats after exhaustive exercise.

GW25-e3110

Salvianolic acid B Suppresses Activated Platelets Induced Inflammatory Cytokines mRNA Expression in Endothelial Cells

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Objectives: Salvianolic acid B (SAB) is a hydrophilic component isolated from the Chinese herb Salvia miltiorrhiza (Danshen), which has been clinically used for the treatment of ischemic cardiovascular and cerebrovascular diseases. In this paper, we focus on the modulating effects of SAB on inflammatory reaction in endothelial cells triggered by activated platelets.
Methods: Human umbilical vein endothelial cells (EA.hy926) were pretreated with SAB (final concentration1, 5, 10μg/ml respectively) for 24h followed by co-cultured with ADP-activated platelets for 12h. The adhesion of platelets on EA.hy926 cells were observed by Wright's-Giemsa staining. The NF-κB activation in EA.hy926 cells were detected by immunocytochemistry and a quantitative analysis of phospho-Ser536 NF-κB p65. The inflammatory cytokines mRNA (ICAM-1, IL-1β, IL-6, IL-8, MCP-1) expression in cells were detected by real-time RT-PCR analysis. In addition, to evaluate the inhibitory effects of SAB on platelet aggregation in vitro, the PRP samples were pretreated with SAB (final concentration 62.5, 125, 250, 375, 500, 625μg/ml respectively), then 10μmol/l ADP or 0.4U/ml α-thrombin induced platelet aggregation were monitored. The level of soluble P-selectin released from ADP or α-thrombin stimulated platelets were also detected.
Results: Pretreatment with 10μg/ml SAB could visibly reduce platelets adhesion on cell and a significant decline in NF-κB P65 nuclear-positive cell percentage and, quantitative analysis showed a dose-dependent inhibitory effect of SAB on NF-κB activity. Pretreatment with SAB also decrease the level of inflammatory cytokines mRNA expression of endothelial cells in varying degrees. Specifically, pretreatment with 10μg/ml SAB significantly down-regulated activated platelets-induced ICAM-1 mRNA expression from 12.07 to 5.27 folds and IL-1βmRNA expression from 5.21 to 3.37 folds. The IL-6 mRNA expression was significantly down-regulated from 10.82 to 8.11 folds at 5μg/ml and 4.12 folds at 10μg/ml SAB pretreatment; IL-8 mRNA expression from 7.12 to 4.91 folds at 5μg/ml and to 3.33 at 10μg/ml; MCP-1 mRNA expression from 4.14 to 3.27 folds at 5μg/ml and to 1.86 at 10μg/ml. Additionally, SAB could dose-dependently inhibit ADP or α-thrombin induced platelet aggregation in vitro, the IC50 value was 312.64μg/ml in ADP induced aggregation and 379.74μg/ml in α-thrombin. Significant decline of soluble P-selectin release from both agonists stimulated washed platelets were also observed after samples were pre-incubation with SAB at approximate IC50 value and 2-folds of IC50 values.
Conclusions: In additional to the inhibiting effects on platelets activation, SAB was able to attenuate platelets-mediated inflammatory responses in endothelial cells even if the platelets have already been turned to activated state. This effect was related to inhibition of NF-κB-regulated inflammatory factors expression. Our work may help explain the efficacy of Salvia miltiorrhiza in the treatment of ischemic cardiovascular and cerebrovascular diseases.